Attorney Docket No.: ALX-141 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Yi Wang, Louis Matis and Scott Rollins

Serial No.: 08/236,208

Filed: May 2, 1994

For: METHOD FOR THE TREATMENT OF GLOMERULONEPHRITIS

Examiner : Phillip Gambel, Ph.D.

Group: 1806

Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION OF BERNADETTE L. ALFORD PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Bernadette L. Alford, hereby declare that:

1. I am not an inventor of the invention claimed in the above-identified application, but I am familiar with the application and have read the office action in the application dated July 25, 1995.

2. I received a B.A. in Biology from Marywood College in 1971. I received an M.S. in Biology in 1973 and a Ph.D. in Molecular Biology in 1974 from the Texas University, Denton, Texas. For the past 12 years, I have been involved in the development of new drugs, including monoclonal antibodies, first at Repligen Corporation and subsequently at Alexion Pharmaceuticals. I am now Vice President, Regulatory Affairs and Project Management at Alexion Pharmaceuticals, Inc., New Haven, CT, the assignee of the above-identified application, and have been so since September, 1994.

3. Since 1984 I have been actively involved in the clinical development of new therapeutic agents, including monoclonal antibodies directed against HIV and CD11b, and have been involved in the design of dose ranging and other clinical studies in human patients.

4. I understand that the Examiner has asserted that the specification of the above referenced application "does not adequately teach how to effectively inhibit the disease/treatment endpoint in humans by administering an inhibiting monoclonal antibody." I further understand that the Examiner has asserted that the specification "does not teach how to extrapolate data" obtained from the controlled experimental systems disclosed therein "to the development of effective *in vivo* human therapeutic methods which are directed toward a chronic ongoing disease."

5. With regard to the Examiner's concerns discussed in the preceding paragraph, I believe that the state of the art as of the filing date of the above referenced application and the disclosure of the specification of that application would enable any worker of ordinary skill in the art to develop effective *in vivo* human therapeutic methods for the treatment of glomerulonephritis. This belief is supported by my 12 years of experience in taking new therapeutic agents (and, in particular, monoclonal antibody based therapeutic agents) from the laboratory into the clinic and my reading of the above referenced application. My specific reasons for this belief are set forth below.

6. In July 1983, the then Office of Biological Research and Review (OBRR, now the Center for Biologics Evaluation and Research - CBER) of the Food and Drug Administration (FDA), issued its first "Draft Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use". This Points to Consider document has been subsequently updated on June 1, 1987 and again on August 2, 1994, indicating that, despite earlier difficulties in the development and clinical use of monoclonal antibodies, the FDA recognized as recently as 1994 the therapeutic utility of monoclonal antibodies. A copy of this 1994 revision of the FDA's "Draft Points to Consider in the Manufacture and Testing

of Monoclonal Antibody Products for Human Use" is appended hereto as Exhibit A. This document includes discussions of the design of "Phase I" studies (i.e., initial studies typically involving dose ranging and administration analysis) in human patients (see section IX, pages 26-28), as well as "Phase II" and "Phase III" studies (see sections X and XI pages 29-31), which typically involve measurements of disease/treatment endpoints.

7. Based upon my experience in designing human clinical studies, it is my opinion that the experimental results presented in the examples of the above referenced application, together with the discussion of dose and administration spanning pages 25 to 28 of that application, would provide sufficient guidance to a knowledgeable worker of ordinary skill in the art to determine appropriate dose range, schedule, and disease/treatment endpoints in human subjects using only routine experimentation. This opinion was arrived at after consideration of many factors, including those discussed in the following paragraphs.

8. The design of initial human clinical trials for any drug will involve certain standard preclinical procedures to determine dosage and administration to be used in initial dose ranging studies in humans. Such procedures typically involve the routine extrapolation of preclinical experimental results obtained *in vitro* and in animal models. Following such initial dose ranging studies, clinical studies of efficacy in humans are designed involving assessment of disease/treatment endpoints. In the specific case of the design of human clinical studies for the treatment of glomerulonephritis using anti C5 monoclonal antibodies (in accordance with the invention disclosed in the above referenced application) appropriate preclinical studies that would allow the routine determination of dose, administration, and disease/treatment endpoints for human clinical studies would include: 1) *in vitro* analysis of complement inhibition in human blood (as discussed in Example 4 of the above referenced application); 2) *in vivo* studies of antibody pharmacokinetics in standard laboratory animals,(as discussed in Example 6 of the above referenced application); 3) animal *in vivo* studies of

antibody efficacy in treatment of disease,(as discussed in Examples 1-3 and 5 of the above referenced application); and, if possible, 4) *in vivo* studies of antibody pharmacokinetics in primates (i. e., monkeys, as discussed in the "Declaration of Scott A. Rollins," submitted herewith).

9. The results of the studies discussed in the preceding paragraph, together with the considerations discussed on pages 25-28 of the above referenced application, allow the design of initial clinical dose ranging studies in human subjects by simply extrapolating the information obtained in animal studies regarding effective dose and administration parameters in light of the *in vitro* data obtained using human blood. Such extrapolations are routine in the art and can be readily performed by those of ordinary skill. The determination of disease/treatment endpoints is similarly routine, given the animal and *in vitro* data available.

10. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Bernadette L. Alford

Bernadette L. Alford, Ph.D.

Signed at New Haven, Connecticut
this 11 day of April, 1996.

Exhibit A

Draft Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1994)

Center for Biologics Evaluation and Research, FDA

Table of Contents

Introduction

The Center for Biologics Evaluation and Research (CBER) is revising the 1987 "Points to Consider (PTC) in the Manufacture and Testing of Monoclonal Antibody Products for Human Use". This updated version is designed to assist sponsors and investigators regarding monoclonal antibody (mAb) product development, including information submitted when filing Investigational New Drug Applications ("INDs") and Product License Applications.

This document supersedes the 1987 document and reflects the considerable experience that has been gained and discussed at several national and international meetings. These meetings include:

1. FDA's Vaccines and Related Biological Products Advisory Committee, which met in August 1990 to discuss "Potential Retroviral Contamination of Biological Products",

2. "Virological Aspects of the Safety of Biological Products", sponsored by the International Association for Biological Standardization (IABS) and held in London in November 1990,

3. "Continuous Cell Lines: An International Workshop on Current Issues", sponsored by FDA, IABS, NIH, the National Vaccine Program Office, HHS, and WHO and held in Bethesda in March 1991, and

4. "Preclinical Safety Testing of Monoclonal Antibodies Workshop", sponsored jointly by FDA and the NIH, held in Bethesda in January 1992.

For mAb, as for other biologicals, existing regulations (21 CFR Parts 200-299 and 600-680) are relevant and should be consulted. In common with the other PTC circulated by CBER, the mAb PTC are not intended to be all-inclusive. When specific products raise special issues that are not considered in these "points", they will be evaluated on a case-by-case basis. For aspects of manufacturing and of the production facility that are not included in this discussion or in applicable regulations, sponsors should consult with the Office of Therapeutics Research and Review and the Office of Establishment Licensing and Product Surveillance as appropriate.

Certain mAb may be designated for lead review by CDER or CDRH in collaboration with CBER. Center jurisdiction is allocated according to the terms of the October 1991 intercenter agreements between CBER and CDER and between CBER and CDRH. These PTC apply to mAb that are reviewed in collaboration according to these agreements.

Filing information

It is not necessary to have all of the information discussed in this PTC complete at the initial IND stage. Rather, the information should be developed during clinical development, with guidance from the appropriate Center by means of frequent dialogue.

The manufacture of mAb that are produced and controlled by similar procedures in the same facility may in some cases be documented in a single Master File. Generally, manufacturing data and information submitted with or incorporated by reference in an IND, Master File, establishment or product license application, are protected from disclosure.

Scope

MAb that are used for *ex vivo* purging of bone marrow to remove immune or tumor cells or that are used in conjunction with devices for *ex vivo* marrow purging, cell collection (e.g. hematopoietic stem cells), or purification of other products should meet the same criteria for purity, potency, safety, and freedom from adventitious viruses as mAb intended for direct administration to patients. Likewise, reagents that are commonly used in conjunction with mAb for *ex vivo* marrow manipulations (e.g. complement) should meet the same standards as mAb intended for direct administration to patients.

Combinations of Monoclonal Antibodies

CBER is open to proposals for clinical testing and licensing of combinations of mAb that are based on a promising pre-clinical evaluation and clinical rationale. Two types of combination mAb products are envisioned at this time: cocktails and panels.

As used in this document, "cocktails" are defined as two or more mAb administered at a fixed ratio. Relevant targets include multiple antigens on infectious pathogens and multiple tumor-associated antigens. The rationale for combining the products should be clear based on the clinical context or clinical data. Lack of interference among the mAb in the combination should be shown and synergistic or additive effects should be characterized. Dose-setting for each of the components is necessary. Dose-setting may be based on preclinical or clinical data that show the necessity or superiority for a particular dose and ratio of mAb in the combination.

As used in this document, "panels" are defined as sets of mAb directed against related antigens (e.g. tumor antigens, HLA types) from which one or more members would be used for an individual patient based on target antigen characterization. Such panels could be submitted for approval in a single product license application. Examples of panels might include anti-idiotype mAb for lymphoma and mAb directed against different bacterial or viral serotypes. Dose-setting for each of the components would be necessary. During the pivotal trials to establish efficacy of the entire panel, some clinical experience with each member of the panel should be obtained.

PRODUCT MANUFACTURE AND TESTING

I. Manufacture of Monoclonal Antibody

At present, most mAb are produced by hybridoma cell lines through immortalization of antibody-producing cells by chemically-induced fusion with myeloma cells. In some cases, additional fusions with other lines have created "triomas" and "quadromas". We anticipate an increase in recombinant and human antibodies in the future. The principles reviewed below may be applied in general to all hybridoma and heterohybridoma generated products, regardless of species of origin. All steps in manufacturing should include current Good Manufacturing Practices (cGMPs).

A. Cell Lines

The following information should be provided:

1. Source, name, and characterization of the parent (myeloma) cell line, including any immunoglobulin heavy or light chains that it synthesizes and/or secretes;

2. Species, animal strain, characterization, and tissue origin of the immune cell;

3. Description of unique immortalization procedures, if any, used in generating the cell line;

4. Identification and characterization of the immunogen;

5. Description of the immunization scheme; in the case of human mAb, any *in vitro* or *in vivo* (e.g.severe combined immunodeficiency [SCID] mouse model) immunization procedures should be described;

6. Description of the screening procedure used; in the case of human mAb, steps performed in order to enrich antigen-specific human B cell populations should be described;

7. Description of the cell cloning procedures (When cells are adapted from serum-containing to serum-free medium, they should be re-cloned);

8. Description of the seed lot system for establishing and maintaining the master cell bank and the manufacturer's working cell bank (MWCB).

See also Cell Lines PTC.

B. Production in Tissue Culture

The following information should be provided:

1. A description of the tissue culture procedures if production is entirely *in vitro* or if cells are passaged *in vitro* prior to mouse inoculation;

2. A description of the tissue culture media used, including certification and testing; (Serum additives used in hybridoma propagation should be free of contaminants and adventitious agents.)

3. The steps taken to prevent or control contamination by viruses, bacteria, fungi, and mycoplasma; and,

4. The acceptance criteria for cells or tissue culture supernatants intended for further manufacture.

C. Production as Ascites Fluid

1. A description of the cell inoculum should be provided (see B.1 above).

2. Animal care should be in accordance with the NIH Guide for Care and Use of Lab Animals. To ensure manufacture of consistent, high quality ascites for production of mAb, an animal health monitoring program should be in place that encompasses quarantine procedures, sentinel animals, and an in-house health surveillance program (including screening for mycoplasma). We also recommend the use of specific pathogen free (SPF) mice. Frequency of serological testing of sentinel mice should be established and is usually based on the prevalence of virus contamination.

3. All protocols for ascites production should also incorporate information on: a) species, sex and age of animals used; b) animal supplier; c) volume of pristane; d) volume and concentration of cell inoculum; e) timing of priming, inoculation, and ascites harvesting; f) frequency and procedure for ascites harvesting; g) definition of "lot"; h) animal bedding, food and water; i) number of animals housed together; and j) environmental conditions under which each procedure takes place.

D. Purification

Purification schemes for mAb should incorporate the following characteristics:

1. Production techniques that will prevent the introduction of and eliminate contaminants, including animal proteins and materials, DNA, endotoxin, other pyrogens, culture media constituents, components that may leach from columns, and viruses;

2. A determination of viral burden prior to purification;

3. Incorporation of one or more steps known to remove or inactivate retroviruses (see Reference 2 and discussion of virus removal validation in Section IV);

4. Validation of the ability of the purification scheme to remove a variety of adventitious agents. We recommend the use in these validation studies of several model viruses encompassing large and small particles, DNA and RNA genomes, as well as chemically sensitive and resistant lipid enveloped and non-enveloped strains. These studies should be performed at the time of implementation of the final manufacturing process.

II. Characterization of Purified Unmodified mAb

Before a mAb is studied in humans, there should be a precise and thorough characterization of antibody specificity, structural integrity, and potency. The mAb should be as free as possible of non-Ig contaminants. A properly qualified in-house reference standard with known structure, specificity, and potency, and that is stored under appropriate conditions and periodically tested to ensure its integrity, should be used for lot-to-lot comparisons. Reference standards should be updated as a product evolves but should be finalized by the start of phase 3 efficacy trials.

A. Structural Integrity

A combination of SDS-PAGE, IEF, HPLC or other appropriate physicochemical methods should be used to show that the purified antibody is not fragmented, aggregated, or otherwise modified (e.g. loss of carbohydrate side chain). Side-by-side comparisons of production lots to the in-house reference standard should be performed.

B. Specificity

Assays should provide evidence that the binding of the mAb to the target antigen is specific. Once the specificity of the antibody is characterized, it should be screened for cross-reactivity with human tissues (see Section VI).

1. Direct binding assays should include both positive and negative antibody and antigen controls. At least one isotype-matched but irrelevant (negative) control antibody should be tested. Negative antigen controls should include a chemically similar, but antigenically unrelated compound, if available (e.g. similar chemical nature, size, charge, and charge density).

2. Whenever possible, the protein, glycoprotein, glycolipid, or other molecule bearing the reactive epitope, should be biochemically defined, and the antigenic epitope itself determined. If the antigenic determinant is a carbohydrate, the sugar composition, linkage, and anomeric configuration should be established.

3. If possible, fine specificity studies using antigenic preparations of defined structure (e.g. oligosaccharides or peptides) should be used to characterize antibody specificity using inhibition or other techniques. For complex biological mixtures, the lots of test antigen and/or inhibitors used for direct binding tests should be standardized. Inhibition of antibody binding by soluble antigen or other antibodies should be measured quantitatively.

4. Once the specificity of an antibody has been determined, it is important to quantitate antibody binding activity by affinity, avidity, immunoreactivity, or combinations of these assays, as appropriate. A number of published methods are suitable for measurement of antibody binding activity (3,4).

C. Anti-idiotype Vaccines

1. In the case of an anti-idiotype vaccine (Ab2 vaccine), the Ab2 immunogen should be characterized as to the Ab2 type, e.g. classical type (Ab2α) or antigen mimic (Ab2β) (5a).

2. Ab2β vaccines should be shown to be reactive with the appropriate population of human Ab1 (antibody to nominal antigen) if such antibodies are available.

3. The Ab2 preparation should be studied for the appropriateness of response (to target antigen) in xenogeneic as well as syngeneic animals (5b).

D. Potency Assays and Specifications

Potency assays are used to characterize the product, to monitor lot-to-lot variation, and to assure stability of the product. Potency may be measured by a binding assay, a serologic assay, activity in an animal model, or a functional assay performed *in vitro* or *in vivo*. It is desirable that the assay bear the closest possible relationship to the putative physiologic activity of the product and be sufficiently sensitive to detect differences in the clinical function of the product. Since potency assays are used to ensure lot-to-lot consistency, they should be reliable, reproducible, and sensitive.

1. Antibody binding activity may be quantitated by ELISA, RIA, radioimmune precipitation, cytotoxicity, flow cytometry, or any other standard, appropriate method. Activity should be expressed as specific antigen-binding activity per mg antibody. Product should be compared to an in-house reference standard. Appropriate measurements of antibody affinity, if established, may be a useful adjunct to other assays. Parallel line bioassay or a similar, valid statistical procedure should be used in calculating potency.

2. The potency of a mAb may also be tested by measurement of *in vivo* function in animal models, although such assays are often cumbersome and difficult to standardize. Animal models can help to validate an *in vitro* potency assay when *in vitro* and *in vivo* measures of activity are correlated.

3. The permissible range of values in potency assays that reflects adequate biological activity of a product should be based on experience with a particular antibody. Ideally, clinical results should be correlated with potency assays in order to develop meaningful *in vitro* surrogate measures of efficacy. This means that a number of production lots should be used during the clinical development program.

III. Special Considerations for Monoclonal Antibodies Conjugated with Toxins, Drugs, Radionuclides or Other Agents (Immunoconjugates)

In addition to previously discussed recommendations for unconjugated (naked) mAb, manufacturers of immunoconjugates should address the following:

A. Construction of the Immunoconjugate.

A full description of the reagents used to construct an immunoconjugate should be submitted, including:

1. The isotype, structure, purity, and binding specificity of the mAb moiety;

2. A description of components such as toxins, drugs, enzymes, and cytokines that are linked to the mAb, including:

a. The source, structure, production, purity (including demonstration of freedom from adventitious agents), and characterization of all components (if components are purchased, a certificate of analysis should be supplied). The toxicity profile of each component should adequately describe the incidence and severity of possible adverse effects;

b. Products derived from cell lines or prepared using genetic engineering methods (e.g., "transfectomas"; bacterially synthesized, chimeric, reshaped, complementarity determining region (cdr) grafted, and single chain Fv antibodies; and recombinant immunotoxins) should conform to recommendations discussed in references (1), (17), and (18).

3. A description of chemical components, such as linkers and chelating agents, that will be used in preparing the immunoconjugate. These should include documentation of the sources of reagents and method of preparation and determinations of residual impurities from synthesis or purification. Charts of the synthetic reaction pathways and any relevant published or in-house data concerning the toxicity of chemicals used in the production of an immunoconjugate should be provided. Reactive intermediates should be inactivated or removed.

B. Purity of the Immunoconjugate

1. Special care should be taken to ensure that the antibody preparations are as free as possible of extraneous immunoglobulin and non-immunoglobulin contaminants as they could react with nuclides, toxins or drugs during the construction of the immunoconjugate.

2. The amount of free antibody and free components in the final product should be determined with limits set for each.

3. The average ratio of coupled material to antibody and the number and location of conjugated moieties per antibody should be determined as the first step in establishing lot release criteria for the final product and developing the relationship between immunoglobulin substitution number, potency, and stability.

C. Immunoreactivity, Potency and Stability of the Immunoconjugate

Coupling of toxin or drug to an antibody may alter the activity of either component.

1. Immunoreactivity before and after coupling should be assessed using appropriate methodology (3, 4).

2. Potency of immunoconjugates, other than for radioimaging, should be assessed.

3. Limits on the percent change in immunoreactivity after construction of the immunoconjugate should be established as part of product specifications.

4. The immunoconjugate should be tested for stability *in vitro* by incubation in pooled human serum at 37°C for at least two half-lives of the product in humans. Aliquots should be analyzed at timed intervals for the concentration of intact immunoconjugate and decomposition products. The conditions

under which product stability is evaluated and the positive and negative controls used should be fully described.

5. The immunoconjugate should be tested for stability *in vivo*.

a. Individual components of an immunoconjugate should be measured during pharmacokinetic and tissue distribution studies in animals and compared to the distribution of unmodified antibody.

b. The target tissues for the various components and the potential toxicities that they may cause should be established.

D. Specific Issues Related to mAb Coupled to Radionuclides

1. The preparation of the radioimmunoconjugate should be performed in a standardized, well-controlled, and validated manner. Methods should be developed to estimate the percent radioactivity in each of the three species of concern: free isotope, conjugated mAb, and labeled, non-Ig substances.

a. It is recommended that the initial IND submission for a radiolabeled mAb contain analytical results from two to three radiolabeling runs that demonstrate the preparation of an immunoreactive, sterile, and pyrogen-free product. The tests should be performed by the same personnel who will conduct the study, using the reagents for the study.

b. Radiopharmaceutical grade isotopes should be used when preparing immunoconjugates. The sterility and pyrogen-free nature of each isotope should be documented by submission of a certificate of analysis and letters of cross-reference to a Drug Master File.

c. The concentrations of covalently-bound and free isotope in the final product as well as residual levels of labeling reagents and their decomposition products should be determined during the trial labeling runs.

d. Quality control tests that will be performed before and after each patient administration should be described.

2. Animal Studies:

a. Animal biodistribution data may be used for pre-phase 1 human dose estimation.

b. Animal models that express the targeted antigen are more likely to reveal antigen "sinks" or tissues with unexpected antigen expression.

c. Xenograft models may evaluate tissue targeting and antigen non-specific radioimmunoconjugate distribution problems but are not helpful at identifying areas of undesired normal antigen expression or tissue cross-reactivity.

d. An adequate number of animals should be studied to achieve radiation dose estimates with an acceptable coefficient of variation (usually less than 20%).

e. There should be complete accounting of administered radioactivity and an adequate number of time points to determine early and late clearance phases.

f. The radioimmunoconjugate should be tested for stability *in vitro* by incubation in serum (see Section III.C.4). Methods should be developed to estimate the percent radioactivity in each of the three species of concern: free isotope, conjugated mAb, and labeled, non-Ig substances.

IV. Quality Control and Product Testing

A. Cell Line Qualification

Qualification of the cell line for production of a mAb to be used as a biologic therapeutic should include screening the master cell bank (MCB) and the manufacturer's working cell bank (MWCB), on a one-time basis, for endogenous and adventitious agents utilizing the tests outlined in Table 1 and described in the Cell Lines PTC. Routine electron microscopy is a useful tool for detecting potential high level virus contamination of cell lines. Because the MWCB is derived from the MCB and propagated for only a few additional passages in tissue culture, abbreviated testing for detecting newly introduced contaminants is acceptable. Any virus contaminant should be identified and quantified in order to establish the extent of virus clearance that the purification process should achieve. When changes in the purification process occur, manufacturers should re-assess viral burden and the need for repeat validation studies. The tropism of any virus for human cells should be determined by appropriate infectivity assays. In the case of tissue culture or fermenter production, end-of-production cells (EPC) should be tested to evaluate whether new contaminants are introduced or induced by the growth conditions. EPC should also be re-examined when there are changes in culture medium or in the scale of production.

Table I
Cell Line Qualification- Tests to be Performed

Tests	MCB	MWCB	EPC
Sterility	+	+	+
Mycoplasma	+	+	+
Virus			
Routine	+	+	+
Species-specific*	+	-	+
Retrovirus**	+	-	+
Authenticity	+	+	+

* Tests for rodent, primate, or human viruses (other than retroviruses), as appropriate

** Retrovirus testing is not required for murine hybridomas; all other cell substrates should be tested

1. Cell lines should be free from bacterial and fungal contamination as demonstrated by sterility testing. Recommended testing procedures for mycoplasma (cultivable and non-cultivable) are described in Reference 1.

2. Screening for adventitious viruses should include routine *in vivo* and *in vitro* tests that are described in Cell Lines PTC.

3. Screening for species-specific viruses (other than retroviruses):

a. The mouse antibody production (MAP) test for murine cell lines (see Appendix 2), the HAP test for hamster lines, and the RAP test for rat lines should be used. *In vivo* testing for lymphocytic choriomeningitis (LCM) virus, including non-lethal strains, is recommended. HAP testing should include minute virus of mice (see III.B.1.c).

b. Material that is contaminated with LCM, reovirus, rotavirus, sendai virus, or Hantaan virus should not be used for mAb production.

c. Monkey cell lines should be screened for the following: herpes viruses (simiae and SA-8), cytomegalovirus (sCMV), encephalomyocarditis virus, simian hemorrhagic fever virus (SHF), varicella virus of simians (sVZV), adenovirus, SV-40, monkeypox, rubeola, and Ebola virus.

d. Human cell lines should be screened for Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B (HBV) and C (HCV), human herpes virus 6 (HHV-6), and any other viruses that are suggested by the medical history of the donor and type of tissue used to establish the original line.

e. For cell lines of other species please consult with CBER.

4. Retrovirus testing of cell lines: Retrovirus contamination of cells from different species varies. The following should be considered when planning experiments to detect retrovirus:

a. Murine cells used to produce monoclonal antibodies should be considered inherently capable of producing infectious murine retrovirus. Therefore, it is not necessary to confirm the presence of murine retrovirus. The amount of retrovirus in the unprocessed bulk, however, should be quantitated on a series of bulk harvests and shown to be consistent from lot to lot (1). Sufficient retrovirus removal by the purification scheme should be demonstrated (see also Section IV.D).

b. Rat myeloma cell lines and hybridomas may not express retrovirus (6). The absence of retrovirus, however, should be demonstrated by co-cultivation of the test article with a cell line(s) susceptible to a wide range of retroviruses combined with a sensitive detection assay, including examination of EPC and several production lots. If retrovirus is not detected by infectivity assays or electron microscopy, further validation studies may not be needed. It is recommended that purification schemes for mAb produced by rat cell lines include one or more retrovirus inactivation or removal steps.

c. Hamster cell lines express defective retrovirus particles (7). Whether hamster cell lines express infectious retroviruses has not been shown. Sponsors should demonstrate the lack of

infectious hamster retroviruses by means of the most sensitive infectivity assays available. These include co-cultivation of the test article with a cell line(s) susceptible to a wide range of retroviruses combined with a sensitive detection assay. As a product moves into phase 2 and pivotal clinical trials, it may be necessary to make additional attempts to detect potential infectious virus by utilizing a wider range of indicator cells, including human cell lines (8,9). Because of uncertainty about the validity of infectivity assays for hamster retroviruses, sufficient retrovirus particle removal by the purification scheme should be demonstrated (see also Section IV).

d. Monkey cell lines should be screened for SIV, STLV, SRV, FV, and HIV and HTLV.

e. Human cell lines should be screened for HIV-1 and 2, HTLV, SIV, STLV, and FV.

5. Authenticity testing should confirm the cell line's species of origin, typical features, and lack of cell-line cross-contamination.

B. Lot-to-Lot Quality Control Monitoring of Unprocessed Bulk Lots and Purified Bulk Lots, and Final Product Specifications

Quality control monitoring should be performed on each lot of product (21 CFR 600.3(x)). This applies to unprocessed as well as purified material.

Table II
Lot-to-lot safety tests to be performed

Tests	Unprocessed Bulk	Purified Product	Final Product
Sterility	+	+	+
Mycoplasma	+	-	-
Virus			
Routine*	+	-	-
Species-specific**	+	-	-
Retrovirus***	+	-	-
Polynucleotide	-	+	-
General Safety	-	-	+
Endotoxin	-	-	+

* In vitro testing with three indicator cell lines should be performed routinely for non-ascites material; in vivo testing is generally done once for non-ascites material but should be repeated when production methods change.
** MAP, RAP, and HAP testing for ascites only
*** Quantitation of retrovirus (infectivity assays or TEM) is important for murine hybridomas; for other hybridomas, TEM, RT, DNA hybridization, and co-cultivation assays are important if MCB or EPC are positive.

1. Unprocessed Bulk Lots

a. There should be no bacterial bioburden in tissue culture harvests. If bioburden testing of pooled ascites harvests shows the presence of viable contaminants, they should be quantified, and allowable limits for bacterial contamination should be set based on manufacturing experience. The identity of the bacterial species should be determined on a periodic basis and whenever the allowable limits for contamination have been repeatedly exceeded. Filtration of ascites harvests through a 0.45 mm filter prior to storage is recommended (see also IV.B.1.b).

b. Tests for cultivable and non-cultivable mycoplasma should be performed on unpurified bulk hybridoma supernatants, prior to any clarification by filtration (1). The filtration of unprocessed bulk ascites through a 0.45 uM filter followed by storage at ≤-60°C prior to testing for mycoplasma is acceptable if positive controls of known titer are processed in a similar fashion simultaneously and the titers are shown to decrease less than 10-fold. If mycoplasma contamination of animal batches or unpurified bulk ascites or hybridoma supernatants is detected, these materials should not be used or processed further.

c. *In vitro* virus testing with three indicator cell lines should be performed routinely; *in vivo* testing is generally done once (as part of cell line qualification, Section IV.A) but should be repeated when production methods change (1). Bioreactors containing hamster cells can become contaminated with minute virus of mice that may escape detection in routine *in vitro* assays. MAP testing for this virus appears to be more sensitive. In all cases, the frequency of monitoring should be specified and justified based on actual experience when using continuous production in contrast to batch production. When contamination with a particular virus is encountered in a facility, consideration should be given to modifying the routine testing program in order to detect that virus.

d. Species-specific virus testing should be performed (see III.A.3).

e. Murine retrovirus contamination should be quantitated routinely for bulk ascites harvests. For tissue culture harvests, retrovirus contamination should be quantitated on several lots in order to establish the level of virus contamination for the specific cell line and manufacturing process (1) (see IV.D). In order to detect non-murine retroviruses, unpurified bulk should be tested using test cell lines that can support the growth of a broad range of retroviruses, including viruses of human and non-human primate origin (1).

f. If ascites production uses different groups of mice, repeated serologic monitoring for species-specific viruses should be performed on each group prior to their use for producing ascites.

2. Purified Bulk Lots

Routine testing on purified bulk lots of unmodified and modified mAb product should include the following determinations (for discussion of immunoconjugates see Section VIII):

a. Chemical purity including the residual amounts of extraneous animal proteins, e.g., albumin, immunoglobulin or other contaminants in the final product. An SDS-PAGE analysis, under both reducing and non-reducing conditions, of increasing amounts of purified material and stained with both Coomassie blue and silver, should be provided. Whenever possible, contaminants should be below detectable levels using a sensitive assay capable of detecting 1 part per million expressed on a weight basis with respect to the mAb;

b. Molecular integrity, including the presence of aggregated, denatured or fragmented product;

c. Immunoglobulin class or subclass;

d. IEF pattern of the antibody (or its heavy and light chains) in each bulk lot with acceptable comparison to the in-house reference standard;

e. Sterility;

f. If infectious agents are identified during the testing of the cell banks (see Section IIIA), the mAb purification scheme should be validated for their removal. The first consecutive 3-5 lots of purified bulk should be tested to confirm that the contaminant was removed by the purification scheme. Should a human infectious agent be identified in the cell bank, every lot of purified product should be tested and consultation with CBER staff is recommended before extensive product development;

g. Lot-to-lot testing for DNA content, prior to any excipient addition, is recommended and the final product should not contain more than 100 pg cellular DNA per dose, if possible (10).

h. Tests for detection and quantification of potential contaminants or additives (e.g., antibiotics, chromatography reagents, preservatives, or components that may be leached from affinity columns such as protein A). Penicillin or other beta lactam antibiotics should be absent. The acceptability of trace contaminants that cannot be removed by standard methods should be discussed with CBER prior to the submission of the IND. Pristane, if used in the propagation of ascites fluid, should be shown to be undetectable by a sensitive test.

3. Final-Filled Product

The following tests should be performed on the contents of final containers from each filling of product (21 CFR 600.3(y)). In certain situations (e.g. user-radiolabeling), special approaches to final container testing may need to be developed on a case-by-case basis after discussion with CBER:

a. Protein concentration;

b. Potency (21 CFR 610.10);

c. Electrophoretic migration of the product in both the native and reduced states on polyacrylamide gels with comparison to the in-house reference standard;

d. Sterility (21 CFR 610.12);

e. General safety test; (21 CFR 610.11);

f. A test for endotoxin; the Limulus Amebocyte Lysate (LAL) assay may be an acceptable equivalent method (21 CFR 610.9) when validated by the rabbit pyrogen test as described in 21 CFR 610.13. A U.S. licensed test system should be used to perform the LAL assay. Comparative testing should be repeated when LAL lots made by a different manufacturer are used. Conditions necessary for comparative testing of the rabbit pyrogen and LAL assay procedures should be discussed on a case-by-case basis. See also "Guidelines on Validation of the LAL Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices", December , 1987 (11).

g. An appropriate identity test (21 CFR 610.14);

h. Moisture (21 CFR 610.13) testing, when appropriate; and

i. Preservative (21 CFR 610.15) testing, when appropriate.

C. Stability of Product

Product stability should meet the demands imposed by the clinical protocol. Accelerated stability testing data may be supportive but do not substitute for real-time data for product approval and labelling.

1. A stability testing program should be developed that includes tests for chemical integrity (e.g., fragmentation or aggregation) and potency at regular intervals throughout the dating period. See also "Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics" (11b). For products that are in clinical trials, significant changes that occur during storage should be reported to CBER. For product license applications, and preferably prior to each stage of a study, stability tests that support the proposed dating period should be performed on the final-filled product, using the container and closure configuration intended for distribution.

2. The stability test for assuring biological activity should include a manufacturer's in-house reference standard. Whenever possible, a single lot of test antigen (e.g. purified antigen, cells or tissue) should be used throughout the study. A quantitative assay should be used to permit a meaningful comparison of activities as defined by potency.

3. Accelerated stability studies help to identify and establish which tests are stability indicating. Important parameters that indicate stability should be monitored by trend analysis on every lot.

D. Quantitation and Removal of a Known Contaminant

The amount of any contaminant in the unpurified bulk product should be estimated. Unpurified bulk mAb, for example, is often contaminated with murine retrovirus. If the contaminant is a virus, any possible or suspected tropism for human cells should be explored (e.g. by co-cultivation assays). This should be followed by demonstration of removal of the contaminant, using the contaminant itself or a

representative analogue of the known contaminant (e.g. a model retrovirus) in a model system purification (1).

Unpurified bulk supernatant concentrates or ascites should be assayed prior to any manipulation other than clarification by low speed centrifugation. We recommend that retroviruses contaminating ascites or supernatants produced by rodent cell lines be quantitated by TEM of concentrated samples of supernatant. Particles that are detected by TEM should be assumed to be infectious for the purposes of establishing target levels of retrovirus removal. If TEM results are negative, it should be assumed that the titer of retrovirus is $1x10^6$/ml. Ascites and supernatants produced by non-rodent or hybrid cell substrates should be assayed by TEM as well as sensitive, state-of-the-art infectivity assays that are able to detect all the expected classes of retroviruses that may be produced by that cell substrate.

Validation that retroviruses are removed or inactivated by the purification scheme is ultimately required. References 1 and 2 also discuss the design of studies to validate retrovirus removal. The goal of such studies is to demonstrate a reduction of virus sufficient to surpass by at least 3 logs the initial level of contamination. An abbreviated approach to documentation of the removal of rodent retroviruses for phase I studies of a mAb is presented under Section V. The design of virus removal/inactivation procedures and process validation should be discussed with CBER during the early stages of product development.

V. Abbreviated Validation Procedures for Certain Phase I Studies

Abbreviated validation procedures should be implemented only after prior consultation with CBER staff with respect to the choice of appropriate alternative virus removal procedures.

A. General considerations

The procedures that follow may be substituted for the current full-scale techniques (1) to demonstrate adequate removal of rodent retroviruses under certain circumstances outlined below. Although these abbreviated validation procedures involve less extensive testing and identification of retrovirus contaminants than the current full-scale methods, quantitation of retrovirus burden in unprocessed bulk product and demonstration of virus removal remain essential to the process.

1. Use of these abbreviated validation procedures is applicable to monoclonal antibodies made in rodent cells, either in ascites or tissue culture, but not to other cell substrates.

2. The abbreviated validation procedures may be used only when the clinical indication is for an immediately life-threatening or severely debilitating disease (causing major morbidity) for which there are no treatments or for which current treatments are unsatisfactory. 21 CFR 312.34 defines immediately life-threatening as a stage of disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment.

3. The recommendations for testing and detection of adventitious agents, as outlined previously in this document and in the Cell Lines PTC, should not be abbreviated.

4. Use of the abbreviated validation procedures will not support studies beyond early phase 1, or studies using healthy subjects or involving large numbers of subjects.

B. Demonstration of retrovirus removal using abbreviated validation procedures

1. General considerations

a. Retrovirus titers in unpurified bulk product (see IV.D) should be used to guide and quantify any purification process.

b. It is recommended that all purification schemes use one or more "robust" virus removal or inactivation steps. Robust steps are defined as those that have been shown to work well under a variety of conditions (e.g. pH or ionic strength of column buffers) with a variety of mAb. Robust steps include low pH, heat, solvent/detergent treatments, and filtration. An estimate of the efficiency of robust steps in removing virus may be demonstrated by: a) a model system ("generic validation"); b) reference to virus removal procedures carried out on a surrogate antibody (generally from the same species and of the same isotype) made by the same sponsor; or c) retrovirus removal studies using the mAb product itself.

c. Affinity and ion exchange chromatography and detergent treatments are not considered robust steps and should be validated for retrovirus removal either by using the mAb product itself or by reference to purification and virus removal using another mAb (surrogate) by the same sponsor. Results for the product and surrogate mAb should meet the conditions for similarity detailed below in section B.3 under Applying the Algorithms.

2. Generic validation is any overall scheme for virus inactivation or removal that can be applied to an entire group or class of molecules. The methods could be cross-referenced in a Master File. Appropriate published supporting data should include only high quality and widely accepted scientific studies. An unpublished method of virus removal based on data from the sponsor's or another laboratory may also be acceptable if the data are judged by CBER to meet high standards for scientific quality.

3. Applying the algorithms:

a. The surrogate mAb used in a generic virus removal validation procedure should be similar to the product Ig mAb in class/isotype/species (e.g. IgG v IgM), charge, and other major biochemical features. In general, the surrogate mAb will be of the same isotype and from the same source as the product antibody (e.g. both from ascites or hybridoma/transfectoma supernatant).

b. The virus removal and inactivation steps used in the generic virus removal system should be similar, if not identical, to those used in purifying the product mAb. Particular attention should be paid to column elution buffer conditions, including pH and ionic strength, the sequence of columns, protein concentration, dwell times, pressure conditions, temperature, and potential problems associated with scale-up, at all steps of virus inactivation and clearance. Similarity of purification and virus removal steps should be supported by data obtained from the sponsor's laboratory for the product validation.

4. Other considerations:

a. Complete or full-scale validation data demonstrating adequate retrovirus removal (see section IV.D) should be provided when the product enters expanded phase 1 or phase 2 testing. This will involve demonstrating that the purification procedure for the mAb, itself, removes any known or highly suspected virus contaminant (1), by using the actual antibody product in a simulated purification and showing the contribution of each step to virus removal or inactivation.

b. Informed consent documents for phase 1 studies of mAb produced using the abbreviated validation procedures need to reflect accurately the potential risk of inadvertent murine retrovirus infection in humans.

c. The abbreviated validation procedures do not apply to antibodies destined for patients with known retrovirus infections (e.g. HIV-1 and 2, HTLV-1 and 2) because of concerns about the potential interaction between murine and human retroviral sequences, or to those with substantial cellular immune deficiency.

d. Table III shows ranges of retrovirus removal (expressed in logs) that might be expected with various robust and non-robust inactivation/removal steps and is presented to aid manufacturers in the design of mAb purification schemes. Claims for the efficiency of retrovirus removal for any particular step will vary depending on a variety of factors and should therefore be based on actual experience, taking those factors into account.

Table III [2]

Inactivation Step	Robust	Reported log virus removal
pH 3-4.5	yes	3-4
heat	yes	4
solvent/detergent	yes	5
filtration (.02-.04 micron)	yes	4-8
detergent	no	4
affinity/ion exchange	no	1-5

VI. Issues Related to Manufacturing Changes (Demonstration of Product Equivalence)

A. Manufacturing Changes During Clinical Development

Changes in the product manufacturing scheme frequently occur during clinical development of mAb. Sponsors should anticipate these changes and develop a plan for demonstrating that the products made by the old and new schemes are equivalent, particularly when preclinical or clinical data developed prior to the production changes will be used to support further clinical trials and marketing applications. Plans for demonstrating product equivalence should be submitted in a timely manner to CBER for review and approval.

Rigorous *in vitro* biochemical and functional characterization of mAb should always be performed whenever changes in manufacture occur (see Product Manufacture and Testing, Section I). Depending on the type of *in vitro* assays and animal studies and quality of the data, extensive clinical data demonstrating equivalence may not be necessary. The need for a more extensive formal comparative clinical evaluation of the product produced by two different manufacturing schemes is increased in certain situations:

1. Product activity cannot be adequately characterized by analytical testing.

2. Biochemical testing shows differences in the products.

3. Animal testing reveals pharmacokinetic or other differences in the products.

When changes in manufacturing occur during early clinical development, evaluations of clinical equivalence should be incorporated into ongoing clinical trials. When changes in manufacture occur late (phases 2/3) in clinical development, additional clinical evaluation may be requested if biochemical and functional characterization of mAb indicate that the older and newer products differ. The design and extent of these trials will depend on the question(s) being asked. For example, if concerns relate to altered clearance rates, then comparative pharmacokinetic studies may suffice. In all cases, comparative studies in the intended patient population should have the appropriate design, including adequate statistical power, to address the particular question (12). With careful planning, many of these studies can be integrated into the overall drug development program for the mAb

B. Manufacturing Changes Subsequent to Product Approval

When significant changes are to be made in the cell banks or manufacturing of a marketed product, the manufacturer should submit a product license amendment including data providing assurance that the information in the product label adequately describes the product made by the new scheme. Often biochemical and functional characterization of the mAb produced by the old and new procedures and repetition of adventitious agent testing and virus removal validation studies will suffice. In other cases (for example, A.1-3 above), comparative clinical trials will be necessary (12). Sponsors should develop a proposed trial keeping in mind biochemical and animal testing results as well as statistical considerations and are encouraged to submit a draft for CBER review and comment.

PRECLINICAL STUDIES

VII. Testing Cross-Reactivity of Mab

When the same or related antigenic determinant is expressed on human cells or tissues other than the intended target tissue, binding of the antibody to this tissue may be observed. Cross-reactivity may have serious consequences, particularly when pharmacologically active antibodies or cytotoxic immunoconjugates are used. Accordingly, laboratory tests should always be conducted prior to phase I to search for cross-reactions. In the special case of bispecific antibodies, each parent antibody should be evaluated individually, in addition to testing of the bispecific product.

A. *In Vitro* Testing for Cross-Reactivity

Human cells or tissues are presently surveyed immunocytochemically or immunohistochemically. Appropriate newer technologies should be employed as they become available.

1. Reactivity of the antibody or immunoconjugate should be determined with the quick-frozen adult tissues listed in Appendix 1. Surgical samples are preferred. Post-mortem samples are acceptable with adequate tissue preservation. Tissues from at least three unrelated human donors should be evaluated in order to screen for polymorphism. The effect of fixatives on tissues that are known to be positive should be evaluated to ensure that the target antigen is preserved during tissue processing.

2. In special situations it may be appropriate to assay cross-reactivity on representative cultured cell lines, stem cells, and embryonic/fetal tissue.

3. Several concentrations of the product should be tested. The ability to detect cross-reactions may depend on antibody concentration. Antibody affinities as well as expected achievable peak plasma concentrations should be considered when choosing the proper concentrations for tissue binding studies. An attempt should also be made to compare the ratio of target to cross-reactive tissue binding. Because non-specific binding may be observed, the specificity of potential cross-reactions should be assessed using inhibition assays with purified antigen, when available.

4. Positive and negative controls are essential for interpreting study results. Controls confirm the acceptable condition of the tissues and adequacy of the assay. Anti-transferrin receptor mAb may be a useful positive control, since transferrin is a common and abundant molecule on the surface of growing normal and tumor cells.

5. If a derivatized antibody or antibody fragment is to be used clinically, it should be tested in that form if at all feasible. The substitution of antibodies of similar specificity for cross-reactivity testing is discouraged.

6. When cross-reactions are encountered and there is a reason to suspect polymorphism of the target antigen, studies should be expanded to a larger panel of tissues to ascertain their frequency.

7. A comparison of *in vitro* cross-reactivity in animal tissues from different species is important in determining the most relevant animal for subsequent toxicology studies.

B. In Vivo Testing for Cross-Reactivity

Cross-reactivity of a monoclonal antibody with non-target human tissues should indicate a comprehensive *in vivo* investigation, first in animals and subsequently in humans. This finding, particularly with cytolytic immunoconjugates or antibodies with ADCC activity, generally mandates more extensive preclinical testing, including studies in more than one animal species and repeat dose animal studies.

VIII. Preclinical Pharmacology, Safety, and Toxicity Testing

Preclinical safety testing of mAb is designed to identify and estimate the incidence and severity of potential adverse events in humans and to identify a safe starting dose and dose escalation, when possible. Preclinical testing concerns surrounding mAb products include their immunogenicity, stability, tissue cross-reactivity, and effector function(s). Species differences may complicate the design and interpretation of preclinical studies.

A. General considerations:

1. Changes in manufacturing or formulation may result in significant changes in biological activity. Therefore, it is recommended that the material used in the preclinical studies be manufactured using the same procedures as the lot or batch intended for clinical trials. In some cases it may be appropriate to modify the components of the delivery system for preclinical testing. For example, substitution of the homologous animal serum albumin for human serum albumin that is used as a carrier will prevent the formation of anti-albumin antibodies in animal studies and thereby increase the validity of preclinical testing.

2. To the greatest extent feasible, preclinical testing schemes should parallel those proposed for clinical use with attention to dose, concentration, schedule, route, and frequency. The range of doses selected for study should include at least one dose that is equivalent to and one dose that is a multiple of the highest anticipated clinical dose. For safety testing, a broad dose range should be explored to ensure that differences in distribution and elimination that may occur at the highest doses do not result in adverse effects. For toxicity testing, the highest doses tested should elicit adverse effects. Dose ranges are generally best established with a minimum of three doses. The linearity and overall shape of the dose response curve should also be defined by investigation of several doses and dosing intervals.

B. Animal Safety Studies

When planning toxicity testing for mAb, the following should be considered:

1. If the test article is a naked antibody and there is no animal model of disease activity or animal that carries the relevant antigen, and cross-reactivity studies with human tissues are negative, testing may be as limited as the General Safety Test (21 CFR 610.11).

2. When an animal model is available, an attempt should be made to demonstrate dose-dependence of biological effects. The use of higher doses may allow a better prediction of the therapeutic index.

3. *In vivo* activity models have proven valuable in providing a rationale for the proposed product use and in defining safety and toxicity. For example, xenograft models can be used to evaluate the binding of antibodies to human tumors. Animal disease models are available to study the effects of mAb on many inflammatory and autoimmune diseases, and allograft rejection.

4. The properties of a relevant antigen in the animal should be comparable to those in humans in biodistribution, function, and structure. For example, studies of $CD34^+$ progenitor cells in the baboon are useful because the same cell fractions in both species express the $CD34^+$ antigen and produce hematopoietic engraftment. Absolute equivalence of antigen density or affinity for the mAb, however, is not necessary for an animal model to be useful. Differences in binding, for example, may be compensated for by increases in the dose or dosing frequency. Differences between the animal and human in antigen number, antigen affinity for mAb, or cellular response to mAb binding, should be quantified. This will allow more accurate extrapolation of safe human starting dose(s) and estimation of the margin of safety.

5. Immunoconjugates containing radionuclides, toxins, or drugs should undergo animal toxicity testing because of possible conjugate degradation or activity in sites that are not the result of mAb targeting. Testing should include the toxicity and target organs of the individual immunoconjugate components including free toxin, drug or nuclide. Results should be correlated closely with studies of conjugate stability. Studies of the immunoconjugate should be performed in a species with the relevant target antigen or disease model, whenever available. Toxicity testing of free toxin or nuclide may be performed in a different species.

6. Routine assessments of mutagenicity are not generally required for mAb.

C. Pharmacokinetics/Biodistribution

A pharmacokinetic model may aid in the interpretation of preclinical activity and toxicity, and in the recommendation of an appropriate dosing regimen and thereby improve the design of clinical trials. Such studies should aim at determining pharmacokinetic and pharmacodynamic endpoints. Studies of biodistribution may provide the initial evidence for inappropriate tissue targeting by a mAb or explain toxicities that are observed in animals. Interpretation of data should consider species of origin, isotype, whether the mAb is an intact immunoglobulin or a fragment, method of labeling, stability of the immunoconjugate, level of antigen expression in the recipient, binding to serum proteins, and route of administration. MAb half-life is also affected by glycosylation, susceptibility to proteases, presence of circulating antigen, and host immune response. The presence of antibodies to the product may alter biodistribution, metabolism, and clearance.

1. Selection of the animal species for testing should be guided by the following considerations:

   a. Preference should always be given to study of a mAb in an animal model in a species that shares a cross-reactive or identical target antigen with humans. For naked mAb directed at human antigens, as opposed to foreign antigens (bacterial, viral, etc.), studies in animal species lacking the target antigen may not be necessary unless they are designed to address manufacturing issues (see Section VI on Product Equivalence).

b. Study of non-human primates is appropriate for unconjugated mAb when there are antigen binding data that indicate that primates are the most relevant species.

c. Normal rodent and murine xenograft models should be critically evaluated for their likelihood of predicting accurately human pharmacokinetic behavior of mAb.. Xenograft models may be more useful in evaluating the ability of mAb to bind to human tumors *in vivo*.

2. Pharmacokinetic parameters should be defined by more than one method (e.g., a radiolabelled mAb should be assayed by ELISA and measurement of radioactivity). In the case of immunoconjugates of any type, intact conjugate should be distinguished from free mAb and free ligand (e.g. toxin, drug, or radionuclide).

3. The development of anti-immunoglobulin antibodies greatly complicates study and interpretation of the effects of repeat dosing in animals. Murine antibodies are non-immunogenic in mice but are immunogenic in humans, making it difficult to extrapolate the results of repeat dose studies in mice to planned repeat dose administration in humans. The reciprocal problem will occur with fully human or "humanized" mAb. Repeat dose studies in rodents in this case may be of little value.

4. Pharmacokinetics or pharmacodynamic studies may be of utility in the demonstration of product equivalence when changes in manufacture occur during product development (see Section V on Product Equivalence). Pharmacokinetic parameters that are useful for determining product equivalence include T_{max}, C_{max}, and AUC calculated from the product time-activity curves in the appropriate animal species.

CLINICAL STUDIES

MAb administered to humans have usually been well-tolerated. Rare instances of serious or fatal adverse events have generally resulted from unintended binding of mAb to specific antigens. These rare events thus emphasize the importance of the tissue screen for mAb cross-reactivity, particularly when relevant-antigen animal models are not available, in order to alert physicians to potential toxicities and direct more conservative dosing schemes.

IX. General Study Design for Phase 1

A. Goals

Phase 1 studies should: a) determine common toxicities; b) explore the relation between dose and toxicity; c) determine the pharmacokinetic behavior of the product in humans; d) collect data on immunogenicity; and, in some cases e) seek preliminary evidence of activity and its relation to dose.

It should be recognized that antibody-specific side effects are more likely to occur with antibodies containing an Fc portion that can activate complement or effect ADCC (e.g., human IgG1 and IgG3 and mouse IgG2a), leading to lysis of bound cells. MAb may also cause desired or adverse effects by blocking or inducing functions of target cells (e.g. lymphokine release syndrome following triggering of T-cell receptors by anti-CD3).

MAb fall into several broad categories. Each of these may warrant a slightly different approach to study in phase 1. In the case of therapeutic unconjugated mAb it may not be necessary or possible to continue studies to the point of the Maximum Tolerated Dose (MTD) or Dose-Limiting Toxicity (DLT). Instead, determination of an optimal biological dose (OBD), defined by the degree of antigen binding or saturation or achievement of the intended biological effect, may be a more appropriate goal. Immune activation, when relevant to the mechanisms of action or toxicity of the mAb, should be estimated. In the case of radiolabeled therapeutic mAb or immunotoxins, undesired tissue targeting and premature release of conjugate are major concerns. Patients receiving immunotoxins need to be monitored for capillary leak syndrome and liver, kidney, and muscle injury.

B. Study Population

1. In general, subjects in clinical trials of mAb should be representative of the population that will be targeted for eventual product use. The potential immunogenicity of most mAb studied to date and the usual paucity of detailed toxicity data in healthy animals that possess the target antigen or in an animal disease model mean that healthy volunteers are generally not appropriate candidates for phase 1 trials. When healthy volunteers are considered for the initial studies of a mAb, the informed consent should reflect the absence of direct medical benefit and the potential immediate and long-term risks of receiving xenogeneic proteins. These include possible toxicity, allergic reactions, and future inability to receive diagnostic and therapeutic mAb because of the development of an immune response (e.g. human anti-mouse antibody or HAMA; note that the use of the term "HAMA" throughout this discussion refers to antibody to any xenogeneic immunoglobulin). Situations in which healthy volunteers might be used in phase 1 are:

a. When the risks of studying a new agent in the index population are too high- e.g., when the index population expresses abnormally high levels of antigen, raising specific toxicity concerns, or when the index population may be particularly vulnerable to toxicity such as neonates.

b. When the index population is too ill to give interpretable safety data- e.g., patients with sepsis.

2. Sponsors and investigators should carefully consider whether single administration, multiple administration in a single course, or multiple courses of therapy will form the basis of the treatment strategy with greatest potential. The route and timing of mAb administration that ultimately will be used in clinical practice need to be considered in an appropriately designed phase 1 trial(s). The effects of intercurrent therapies or repeat administrations of the mAb may alter safety and efficacy. Changes in antigen mass (due to binding and clearance or antigen modulation by the mAb) and immune responses to the mAb, for example, may mean that single dose data cannot be extrapolated to multiple dose schedules. Furthermore, repeat administrations in the face of HAMA may lead to toxicity and loss of therapeutic benefit. Modified dose schemes to compensate for high HAMA or circulating antigen may need to be studied, including pharmacokinetic studies to determine the relationship between HAMA titers or circulating antigen levels and organ distribution, clearance, and toxicity.

3. Patients pre-exposed to xenogeneic proteins or with a history of xenogeneic protein allergies should be excluded from phase 1 studies of mAb products derived from the homologous species.

C. Dose-setting

Whenever possible, the selection of the phase 1 starting dose should be based on safety and toxicity information from testing in a relevant animal model. In extrapolating from animal doses to human doses, information about relative affinity of the mAb for the human antigen and its animal analogue may be of great value. Whenever animal studies are judged to be impossible or of no relevance and initial studies are in humans, testing should begin at a low dose that is based on extrapolation from tissue culture studies or from trials of similar mAb. The target *in vivo* dose or concentration range should be based on *in vitro* studies of cells for which antibody-antigen affinity and functional activity (immune modulation, cytotoxicity) have been measured, combined with study of a relevant-antigen animal model to assess *in vivo* activity, if available.

1. Single dose studies

a. Initial studies of therapeutic mAb in phase 1 should be dose escalation studies. The goal should be to define the maximally tolerated dose (MTD) and/or an optimal biologic dose (OBD) that is defined by a surrogate laboratory measurement (e.g. receptor binding or target blood level). Toxicity and effectiveness criteria should be disease and indication specific. It is prudent to select a starting dose that is lower than the dose that is estimated to be biologically active. In most studies a new population of patients is used for each dose to be tested. A variety of dose escalation schemes are in widespread use (13-15).

b. Studies of radioimmunotherapeutic mAb should employ a traditional dose escalation format with the lowest and highest doses based on animal dosimetry and projected normal organ radiation tolerance.

c. Stopping and dose modification criteria for toxicity should be explicitly defined. For non-life threatening or chronic diseases, the protocol should specify dose reduction when any significant toxicity is encountered in contrast to trials of anti-cancer agents and other drugs for life-threatening diseases in which a higher tolerance for toxicity is often appropriate. In any disease, the occurrence of an unexpected toxicity should prompt reconsideration of the dose escalation, exclusion criteria, informed consent, etc. The safety assessment should include relevant laboratory as well as clinical adverse events. Long-term safety follow-up is often necessary for assessing potential reactions, including monitoring for completeness of restoration of immune function, and should be specified in the protocol.

2. Multiple dose studies

a. The development of less immunogenic antibodies may facilitate repeat dose applications.

b. If a multiple dose regimen of a mAb is anticipated, multiple dose schedules should be explored in phase 1, after basic data on peak levels, clearance, and toxicity are available from the single dose studies. The time required for recovery from the biologic effects (e.g. immune recovery after $CD4^+$ cell depletion or modulation or return of bone marrow function after radioimmunotherapy) of single doses should also be well understood prior to multiple dose regimens. Whatever patterns of multiple dose schedule are envisioned, the rationale should be provided based on dose tolerance and available pharmacokinetic and pharmacodynamic data in humans and/or animal models.

c. For repeat administration of a radioimmunotherapeutic or immunotoxin, the investigator should identify with great precision all organ toxicities and pathology. The length of time that it takes for recovery from all toxic effects to occur should be monitored. Intra-patient dose escalation is generally inappropriate because cumulative toxicity, especially to bone marrow, may occur and it may be difficult to determine whether effects are due to prolonged therapy or to increased dosing levels. Intra-patient dose escalation may be appropriate if no toxicity is seen at the initial dose levels or when individual variation in tolerated dose is large suggesting a role for an initial safe "test" dose(s). Dose escalation within patients should consider threshold and carryover effects as well as reversibility of clinical and laboratory adverse events to baseline values.

3. If any adjunctive therapies are proposed for routine use in conjunction with the mAb, their dose and any potential interactions should be studied in phase 1.

D. Pharmacokinetics

Design of pharmacokinetic studies should consider the species in which the immunoglobulin is produced, the immunoglobulin class and subclass, and the structure of the antibody (e.g. whole mAb, Fab fragment, etc.) or immunoconjugate. A study population with the appropriate antigen and antigen mass is of great importance; such studies may be stratified by antigen mass. Pharmacokinetic studies

may also be very useful in demonstrating equivalence of different products or formulations (see Preclinical Section VIII). Pharmacokinetic studies optimally include:

a. Determination of plasma concentration and clearance;

b. Determination of optimal *in vivo* dose based on correlation with effective concentration estimated from *in vitro* studies;

c. Determination of peak and trough levels and elimination rate constants to facilitate design of multi-dose regimens;

d. Determination of organs used for clearance;

e. Monitoring of immunoconjugates by assaying the fate of the whole molecule and its components; and

f. Investigation of relationship between clearance and method of administration, antigen load and circulating antigen, and HAMA.

X. Phase 2 Studies

When phase 2 studies begin, the antibody specificity should be characterized to the fullest extent possible.

A. Goals

A series of well-designed phase 2 studies are of great value in developing definitive and successful phase 3 trials. During phase 2:

1. The most reliable measure(s) or endpoint(s) for determining clinical utility should be explored and established.

2. Dose range, route, schedule and other technical considerations related to product administration should be well-explored by the completion of phase 2.

3. Preliminary estimates of the appropriate phase 3 trial size should be developed, based on estimates of the size of the therapeutic or diagnostic effect.

4. Populations that are most likely to show beneficial or adverse reactions to the mAb should be identified.

5. Considerable data regarding adverse reactions should be accumulated.

6. Mechanisms of action (pharmacodynamics) should be explored through measurements of laboratory endpoints and correlation of these with routes of administration, dose schedules, clearance, antigen load, and HAMA.

B. Study Population

1. Patient populations that differ from those studied in phase 1 (e.g. different stage of disease, impaired organ function, tumor bulk, performance status, duration of illness, age, bone marrow reserve, pre-existing immune suppression, concomitant non-mAb therapies, etc.), should be studied in phase 2, particularly if it is likely that these populations will be studied during phase 3 or included in the labeled indication. If the patient population differs significantly from that studied in phase 1, additional pharmacokinetic data may be useful. Clearance under conditions of organ impairment (e.g. renal and hepatic) should be studied when appropriate.

2. Further safety investigations should include evaluation of specific organ toxicity, and safety of repeat dosing (e.g. in the presence of HAMA).

3. Product development of many agents may require treatment algorithms individualized by patient. Attention should be paid to when in the course of an illness mAb therapy is indicated or beneficial, how it interacts with conventional therapies, and factors that predict individual patient responsiveness to treatment. Phase 2 provides the opportunity to identify and test the appropriate treatment algorithms for eventual incorporation into phase 3 trials and final labeling.

C. Dose-Setting

Phase 2 studies should evaluate a variety of doses and schedules of administration in a diverse and representative patient population. Biologic or biochemical assays should be available to determine the blood level of the product and/or its activity. Differences in disease severity or in patient demographics may mandate different treatment regimens. Different doses should be evaluated in sufficient numbers of patients in each study group. Surrogate markers of efficacy may be particularly helpful in guiding dose or schedule selection.

D. Randomization

Patient randomization is of value in comparative dose-finding trials, in evaluating the utility of cold antibody to clear circulating antigen or block non-specific mAb binding, in defining the correct cold antibody dose, or in determining the importance of drug schedule or route of administration. In many cases it is desirable that such phase 2 trials be double-blinded as well as randomized to avoid misleading results that can have adverse consequences for the planning of successful phase 3 trials.

E. End of phase 2 meeting

Consultation with CBER at the end of phase 2 in preparation for efficacy trials is strongly recommended. A meeting is often advisable. Prior to such a meeting, sponsors should submit to CBER:

1. A well-organized summary of the clinical data, including a summary of the existing safety database and effect of dose-response on toxicity and a summary of the data on clinical activity;

2. Complete information on product characterization, including the potency assay(s);

3. Information demonstrating that a facility and manufacturing methods that can produce sufficient product to complete the Phase 3 study and support initial commercial marketing are in place or under consideration;

4. Information on any contract, joint and divided manufacturing arrangements;

5. Analytical techniques and production SOPs to demonstrate adequate consistency across lots during the phase 3 trials;

6. Information on products that will be used in conjunction with the mAb, such as rabbit complement; this information could be submitted in the form of a Master File.

XI. Phase 3 Studies

A. Goals

The results of phase 3 trials should provide the definitive efficacy and safety database for product approval.

B. Study Population

Any group that will receive the mAb once it is marketed should be adequately represented in the phase 3 trial study populations. Inclusion of a sufficiently diverse patient population is particularly important if there are scientific reasons to suspect that mAb biodistribution, metabolism, or side effects may differ because of gender, age, or racial differences.

C. Dose-setting

Every effort should be made prior to phase 3 trials to identify an active dose range that has an acceptable toxicity profile, using surrogate or clinical endpoints. In the phase 3 trials, it is frequently desirable to design trials with 2 or more doses or schedules.

D. Product issues for phase 3

1. Study drug should be manufactured using the procedures, scale of production, and facility that are intended to be used commercially.

2. Scale-up programs and contemplated changes in product manufacture should be addressed before phase 3 trials. If major manufacturing changes (e.g. change of cell bank, change from serum-containing to serum-free medium, or change of site or portion of site of manufacture) are made after the phase III trial is completed, clinical studies may be necessary depending on the information yielded by pre-clinical biochemical, pharmacokinetic, and functional comparisons (see Section V).

3. Sponsors should study a number of separate product lots in phase 3 in order to demonstrate the ability to prepare reliably a safe and effective product.

XII. Immunogenicity: Clinical Considerations

Monitoring of antiglobulin titers and immune activity is of great importance in evaluating the safety and efficacy of mAb and in designing protocols involving retreatment. The consequences of an immune response to mAb include interference with the efficacy of a mAb and a variety of potentially dangerous immunological reactions.

A. Monitoring the Development of Antibodies to mAb

Assays for HAMA have been well-studied. Depending on the source of the mAb, assays for other anti-immunoglobulins will need to be developed to detect human anti-rat antibodies (HARA) and human antibodies directed against chimeric (HACA), humanized, and primatized antibodies, immunonuclides and immunotoxins, their individual components (e.g. ricin), and neoantigens formed by the linked antibody/toxin/nuclide.

1. The timing of sample collection for HAMA testing should take into account whether the intended use of the mAb is for single or multiple administration. HAMA titers should always be established at baseline to account for pre-existing antibodies (including anti-globulin or anti-conjugate antibodies, when appropriate) and before readministration of a mAb. Post-administration samples may be drawn early (e.g. 2 weeks post administration), but should also be drawn at a later time point (e.g. 6-8 weeks).

2. Any selected HAMA assay(s) should be standardized to the extent possible. A "standard" anti-mouse antibody preparation of defined specificity from a human or primate source should be selected and then aliquoted and frozen. This will facilitate future intra- and interstudy comparisons. The "standard" can also be used to establish a linear standard curve for routine testing. Specificity should be established by appropriate testing employing inhibition or competition studies and negative and positive controls. There should also be studies of the range of reactivity of normals and evaluation of the potential interference by serum components such as bilirubin and lipids. The sensitivity, specificity, precision, and accuracy of the assay should be established,

4. The specificity of the patients' immune responses to the mAb should be identified and characterized, i.e. whether the responses are generated against heavy chain isotype or light chain, to constant (C) region, variable (V) region or idiotypic epitope(s), or immunoconjugate or neoantigen. These data will demonstrate whether it is possible to use a HAMA test with broad specificity (detecting human antibodies reactive with both heavy-and light chain constant region antigens of all mouse immunoglobulin classes), or whether a more restricted HAMA test that is idiotype-specific is necessary. In certain instances it will be necessary that the HAMA assay use the mAb product as the detection antigen.

5. The choice of the appropriate HAMA assay depends on the proposed use and labeling of the product. Development and validation of a HAMA assay should accompany the clinical development of a new mAb during the investigational phase. If HAMA responses influence the safety, efficacy, or optimal dosing, the results of HAMA testing should be correlated with product efficacy and adverse events.

6. A PLA for a mAb with an indication for repeat administration should be accompanied by a validated test that reliably measures HAMA responses to the mAb. A commercially available HAMA test kit may be appropriate provided it has been validated for the detection of HAMA which alters the pharmacokinetics, safety, and/or efficacy of the new mAb product. If no appropriate HAMA test is available, a properly validated test system should be developed by the sponsor.

B. Clinical Consequences of Immunogenicity

1. Skin testing is generally not advised because it is a poor predictor of sensitivity to mouse proteins and can cause sensitization.

2. When HAMA is present, adverse events should be anticipated and appropriate precautions taken. Because of the possibility of potentially life threatening reactions, mAb are generally given in facilities where acute resuscitative care is immediately available. The use of non-hospital settings for mAb administration (e.g. clinic or home) should be justified by clinical safety data. Vital signs should be observed closely for at least one hour after completion of the infusion. The possibility of delayed adverse effects from immune responses to mAb should be considered and reflected in the trial design, including appropriate clinical and laboratory testing.

3. Anaphylaxis, anaphylactoid and other immune reactions

a. True IgE-mediated anaphylaxis to whole mouse immunoglobulins is rare. It is theoretically possible that anti-human allotype responses of an allergic nature could occur but they have not been reported to be of clinical importance to date. If the mAb is conjugated to chelating agents or toxins, the likelihood for allergic reactions may be greater. In all cases, repeated administration of a mAb increases the likelihood of hypersensitivity response. Similarly, individuals who have been in contact with mouse proteins may be sensitized. Immediate hypersensitivity reactions may range from mild (e.g. skin rash, urticaria, angioedema, wheezing) to severe (shock, respiratory failure).

b. Infusional reactions such as fever, low back pain, and nausea, are common during or immediately following mAb administration (incidence is approximately 5%). The mechanism of these reactions is not clear, but it may be related to protein aggregates. Frequency and intensity can often be controlled by using a slower infusion rate or by pre-medication schemes.

c. Serum sickness is unusual following mAb administration. Unlike anaphylaxis and infusional reactions, which occur during or immediately after antibody treatment, serum sickness is delayed. High levels of soluble antigen that are present in the circulation should be measured and correlated with any adverse effects of the mAb.

4. HAMA can interfere directly with murine antibody-based clinical tests for antigens such as CA-125 and CEA by binding to the murine detection antibody. Indirect interference with diagnostic assays is theoretically possible if mAb administration induces anti-idiotypic responses that mimic antigens. When appropriate, evidence for either type of interference should be systematically sought using well designed *in vitro* studies. Ideally, attempts should be made to circumvent such interference and any alternative clinical assays should be validated.

5. When a population is selected for testing of a mAb, the risk that future therapy with a monoclonal antibody may be compromised by elicitation of HAMA or other antiglobulins should be considered.

XIII. Dosimetry

Sufficient data from animal or human studies should be submitted to allow a reasonable calculation of radiation-absorbed dose to the whole body and critical organs upon administration to a human subject (21 CFR 312.23(a)(10)(ii)).

A. Calculation of radiation dose to the target organ

Investigators should determine, based on the average patient:

1. The amount of radioactivity that accumulates in the target tissue/organ;

2. The amount of radioactivity that accumulates in tissues adjacent to the target tissue/organ;

3. The residence time of the radioactive mAb in the target tissue/organ and in adjacent regions;

4. The radiation dose from radioisotope, including free radioisotope, and any daughter products generated by decay of the radioisotope; and,

5. The total radiation from bound, free and daughter radioisotopes associated with the radioimmunoconjugate, based upon immediate administration following preparation and delayed administration at the end of the allowed shelf life.

B. Maximum Absorbed Radiation Dose

The amount of radioactive material administered to human subjects should be the smallest radiation dose that is practical to perform the procedure without jeopardizing the benefits obtained from the study.

1. The amount of radiation delivered by the internal administration of radiolabelled antibodies should be calculated by internal radiation dosimetry methodology. The absorbed fraction method of radiation dosimetry is described in two systems: the Medical Internal Radiation Dose Committee (MIRD) of the Society of Nuclear Medicine and the International Commission on Radiological Protection (ICRP) [21CFR 361.1(b) (iv)]. The higher estimate of the absorbed dose determined from either of these systems should be used in the radiation dosimetry safety assessment.

2. Dosimetry estimates should include the following: all target organs/tissues; bone marrow, liver, spleen, kidney, lung, heart, urinary bladder, gallbladder, thyroid, brain, gonads, gastrointestinal tract, and adjacent organs of interest due to known or expected radiation exposure with associated toxicity.

3. Calculations should be provided that anticipate changes in dosimetry that might occur in the presence of diseases in organs that are critical in radioimmunoconjugate metabolism or excretion (e.g. renal dysfunction leading to increased hepatobiliary clearance or hepatic dysfunction leading to increased renal clearance).

4. The mathematical equations used to derive the radiation dose and the absorbed dose estimates should be provided. Sample calculations and all pertinent assumptions should be listed.

5. Calculations of dose estimates should be at the maximum shelf life of the radiolabelled antibody to allow for the upper limit of radioactive decay contaminants and should:

a. Include the highest amount of radioactivity to be administered;

b. Include the radiation exposure contributed by other diagnostic procedures such as roentgenograms or nuclear medicine scans that are part of the study;

c. Be expressed as Gray (Gy) per millicurie of radionuclide; and,

d. Be presented in a tabular format and to include individual absorbed radiation doses for the target tissues/organs and the organs listed in V.C.2.

XIV. Imaging Agents for Cancer

A. Early Clinical Development

Knowledge of the relative proportion of product that binds to target cells compared to background is valuable when deciding which of several candidate antibodies to develop. Phase 1 studies of imaging agents should be performed in a patient population expressing the antigen.

1. Early clinical investigations should collect data on the effects of dose, route and schedule; routes of excretion of isotope, conjugate, and free antibody; *in vivo* stability of the conjugate or chelate; and the influence (on pharmacokinetics) of organ impairment, circulating antigen load, and tumor burden. Where applicable, the effects of the use of cold antibody should be investigated during early clinical development.

2. Radiation dosimetry should be performed with attention to variations caused by the antibody dose. Dosimetry should include internal and excretion dosimetry and enumeration of maximum radiation doses for tumor and different organs (see Section V).

3. Preliminary estimates of optimal imaging times and techniques and required imaging equipment should be made.

4. The ability of the agent to image measurable or occult disease, the smallest detectable lesion, and the quality of imaging in different organs should be assessed.

5. Data should be compared to standard diagnostic techniques whenever possible but should not be used in patient management.

6. Preliminary evidence of image quality is generally sought and serves as the basis for designing later clinical studies.

B. Expanded Clinical Development

Phase 2 trials provide the opportunity to define the appropriate patient population for phase 3 trials and refine technical aspects of the scan. Comparisons of antibody dose should be performed and randomization may be useful at this stage.

1. Phase 2 studies of imaging agents should attempt to define the clinical settings in which the agent will be used once it is licensed. A cancer imaging agent, for example, could be used for screening, initial work-up, detection of occult disease, or staging at relapse. It may be of value only to rule out or rule in cancer or it may be of value for both. Investigators should note that imaging efficacy may be influenced by disease-specific factors (e.g. stage of disease, tumor burden, level of circulating antigen, co-morbid illness) and technical considerations (e.g. timing of imaging, views required for optimizing imaging, equipment, use of cold antibody).

2. Initial evidence of diagnostic validity (e.g. sensitivity, specificity, predictive value) in a variety of settings should be obtained in phase 2 trials. Diagnostic validity of the imaging agent should be confirmed with a "gold standard" test such as histopathology or in some cases conventional diagnostic modalities. When such a standard does not exist, results should be correlated with clinical outcome.

3. Studies in phase 2 should gather sufficient data to allow the generation of hypotheses of clinical utility in specific and carefully identified clinical settings that will be evaluated later in formal efficacy trials. The measure(s) or endpoint(s) most appropriate for the efficacy trial(s) should be identified. Algorithms outlining how imaging agents will fit into the patient work-up should be developed during phase 2 studies.

4. The dose should be more precisely determined in phase 2 by the acquisition of further data regarding the influence of circulating tumor associated or tumor specific antigens, tumor burden, imaging times, and the selection of ratios of hot/cold antibody.

5. Additional data regarding organ dosimetry and adverse reactions should be obtained at the dose and regimen selected for further clinical development.

6. During phase 2, data should be gathered at a number of clinical study sites to show that on-site isotopic labeling can be performed reliably in diverse settings with acceptable sterility, purity, retention of immunoreactivity, and high specific activity.

7. The readministration of monoclonal antibodies for repeated imaging studies should be done only after a product has been properly evaluated for single use with adequate data collection on HAMA development and adverse reactions.

C. Efficacy Studies

1. The following elements should be incorporated into the clinical protocol:

   a. A prospectively defined and detailed clinical efficacy endpoint(s) and analytical plan;

b. A study population consisting of those patients for whom the imaging agent is intended, once approved;

c. A plan for the acquisition and storage of films and/or digitized data for the experimental agent and the test(s) used for comparison; CBER staff should be consulted for technical details of data configuration, transformation, presentation and storage before initiation of studies.

d. Provision for comparisons to standard imaging techniques (if they exist), which are specified in advance. Standard operating procedures for reading all scans should be written. In addition to on-site readings of the experimental and conventional scans, it is recommended that randomly selected scans be analyzed by a blinded independent reader or committee who lack access to the test(s) used for comparison and/or detailed clinical data. When experimental and conventional scans are designed to be read in the context of other clinical data, the exact nature of those data should be specified.

2. A demonstration of clinical benefit should be incorporated in the primary efficacy endpoint of the phase 3 trials. Sensitivity, specificity, and predictive value are not generally suitable primary endpoints by themselves nor are they satisfactory surrogates for efficacy in a phase 3 trial of an imaging agent. Whether sensitivity or specificity will be calculated on a per-patient or per-lesion basis should be specified prospectively and justified based upon the nature of the clinical benefit assessment. In general, clinical efficacy is best assessed on a per-patient basis.

Definition of potential clinical benefits for an imaging agent may include:

a. The primary endpoint demonstrates improved therapeutic outcome: the new test provides a more accurate or timely diagnosis which can be shown or anticipated to lead to longer survival, longer disease-free or symptom-free survival, or a decrease in morbidity;

b. The primary endpoint is improved patient management: 1) the avoidance of potentially morbid surgical procedures is demonstrated because the test provides more accurate information on the extent of disease (e.g. stage) or allows the surgeon to remove more tumor, or shortens operating time; 2) more accurate staging leads to selection of appropriate adjuvant therapies or avoidance of potentially toxic medical therapy if incurable metastatic disease is detected; 3) improved measurement of extent of disease allows anticipation (and possibly prevention) of important complications (e.g. spinal cord compression, superior vena cava syndrome, etc.); 4) the new test finds synchronous primary tumors that are missed by conventional diagnostic modalities (e.g., multiple colon primary tumors, contralateral breast tumors, etc.). Trials aimed at establishing improved patient management may require novel designs. Elements of the design might include:

(1.) The expression of the primary endpoint, whenever possible, as a numerical quantity, with the appropriate confidence intervals (for example, the new test detects occult disease in 10% of patients who have negative assessments by conventional diagnostic modalities; the new test leads to more accurate staging of 10% of patients previously staged by a conventional battery of tests).

(2.) Patient management strategy choices at different points in the work-up and treatment of a disease that may be affected by the information gained from an investigational diagnostic scan should be carefully controlled. Before the investigational test is performed, the assessed likelihood of the diagnosis in question should be specified on data forms that are specific, unbiased, and include instructions as to who completes them, and when in the course of the work-up they are to be completed. Forms should be completed contemporaneously. Additionally, a management plan that would have been followed in the absence of the experimental test should be defined prior to performing the test so that it may be compared to the modified management plan that resulted from the new test.

(3.) The proposed manner in which the new test could be used to alter diagnosis and management should be defined. The protocol should specify whether the hypothesized utility involves use in place of or in addition to other tests (and what those tests are) or if the new test will be used to determine whether another test should be performed.

(4.) Where adverse effects are minimal, an endpoint demonstrating the provision of valid diagnostic information may be acceptable in lieu of demonstrated improvement in patient management. Adverse effects of the new test that should be considered in assessing the risk versus benefit might include patient inconvenience, adverse impact (e.g., HAMA) on future diagnostic and therapeutic interventions, or delay in performing surgery or prolongation of intraoperative time.

c. The primary endpoint is improved patient convenience or comfort: 1) the new test substitutes for a test or a battery of tests that are currently performed leading to improved tolerance and convenience; 2) the new test provides more accurate diagnostic information that leads to shortening of the post-operative period. Use of these endpoints in trials should be discussed with CBER.

d. The test provides unique information that cannot be obtained any other way. Information without direct clinical benefit may be considered as evidence of efficacy if the information is reliable and has intrinsic value to the patient (e.g., defines prognosis or prospects for future therapy), which outweighs any risks of the test.

e. The primary endpoint is diagnostic equivalence to existing tests, particularly when there are advantages in safety, convenience, and/or timing. The equivalence trial should have a pre-specified power to detect inferior diagnostic performance of the new imaging agent. The statistical approach to determining equivalent performance should be described in detail in the protocol.

APPENDIX I

NORMAL HUMAN TISSUES USED IN CROSS-REACTIVITY TESTING

Adrenal
Bladder
Blood cells
Bone Marrow
Breast
Cerebellum
Cerebral cortex
Colon
Endothelium
Eye
Fallopian tube
Gastrointestinal tract
Heart
Kidney (glomerulus, tubule)
Liver
Lung
Lymph node
Ovary
Pancreas
Parathyroid
Placenta
Pituitary
Prostate
Skin
Spinal cord
Spleen
Striated muscle
Testis
Thymus
Thyroid
Ureter
Uterus (cervix, endometrium)

Appendix 2: Mouse antibody production test

The following tests for murine viruses (mouse antibody production test, (16) should be performed on any MCB and EPC derived from murine cell lines and on all lots of mAb derived from mouse ascites fluid.:

Ectromelia
EDIM
GD VII virus
Hantaan virus
LCM virus, including challenge for non-lethal strains
LDH-elevating virus
Minute virus of mice
Mouse adenovirus
Mouse encephalomyelitis
Mouse hepatitis
Mouse salivary gland (murine CMV)
Pneumonia virus of mice
Polyoma
Reovirus type 3
Sendai
Thymic virus

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Note: To obtain Points to Consider and other documents from CBER, please call 301-594-1800.